REMARKS

Claims 1-12 remain in this application.

The Present Invention

An important step in the present invention is to use a combination of two different affinity tags. One of the affinity tags consists of an IgG binding domain of Staphylococcus protein A. Importantly, biomolecule complexes are labeled with two different affinity tags and, due to said tags, can be purified in two different affinity purification steps. Thereby, the biomoleucle complex is first bound to a solid phase via the first tag, whereby impurities which are not bound to said solid phase can be separated in a simple and gentle manner. Subsequently, the biomolecule complexes are separated from the carrier. Thereafter in a second purification step the biomolecules complexes are bound to a second carrier via the second affinity tag. By a second affinity purification, further contaminants can be separated thereby which possibly were bound to the first carrier together with tagged biomolecule complexes and, therefore could not be separated in the first purification step. Biomolecules can then be released from the second carrier as well and, thus, be obtained in purified form. Said basic concept of purifying biomolecule or protein complexes, using at least two affinity tags, one of which contains an IgG binding domain of Staphylococcus protein A, is not described in the prior art.

The nucleic acid sequence is irrelevant to the present invention.

What is claimed is a purification process applicable to biomolecule complexes, the important feature being the use of two tags and the successive performance of two affinity purification steps. Binding to the tags and purification, however, takes place irrespective of the nucleic acid sequence. Accordingly, further recitation of specific nucleic acids is neither appropriate or useful. Any complex can be purified as a person of ordinary skill in the art starting out from a desired complex can easily identify the corresponding nucleic acid. This is true as the genetic code specifies that three bases code for an amino acid in a protein. Starting out from a protein complex to be purified, the person of ordinary skill can easily determine the corresponding nucleic acid sequence with reference to the genetic code. Thus, no undue experimentation is required since a person of ordinary skill can readily determine the relevant sequence at his desk by using the protein sequence information, the genetic code, and optionally know computer programs that can generate sequence information.

The present invention does not relate to nucleic acid sequences and protein complexes per se.

The present invention describes how complexes, i.e. complexes that the user elects, can be purified. Once the user has elected any complex, as described in the present application, two tags and two affinity purification methods are used. Starting out from the elected complex, the skilled person can then easily determine the nucleic acid sequences relevant to the protein sequences of the complex with the help of the genetic code, without any experimentation, simply at his desk.

The Example provides one possible embodiment.

The present invention may be applied by a person of ordinary skill to a variety of areas. If cells other than yeast cells are used, it is necessary, like in the case of yeast cells, to first provide an expression environment containing one or more heterologous nucleic acids encoding one or more subunits of a biomolecule complex. Expression of heterologous proteins in various cells was however state of the art at the priority date of the present invention. As indicated above, this is readily possible because a person of ordinary skill starting out from the protein sequence of the biomolecule complex can easily derive the nucleic acid sequence from the genetic code.

The present application teaches methods and procedures for purifying biomolecule complexs using two different affinity tags and two different affinity purification steps. In the Example describing yeast, specific process conditions are given that can be easily varied by one of ordinary skill. Hence, limitation to the specific conditions in the Examples is not justifiable.

Complexes from more than a yeast host are enabled.

Any biomolecule complexes can be purified according to the invention, whereby the corresponding nucleic acid sequences can be easily determined due to the genetic code. The example contained in the application, wherein a yeast protein complex was purified, however, can be directly applied to other protein complexes. For example, reference is made to the publication by Garvin et al., Nature, vol. 415, 141-147 (2002), specifically Figure 5, wherein it is stated that the protein complexes of yeast have a similar composition to orthologous protein complexes of humans. Accordingly, TAP protein complexes from human and yeast cells could be isolated analogously by the methods of the invention. The Garvin et al. reference shows that when observing the instructions given by the present application it was also readily possible to purify protein complexes other than yeast protein complexes by means of the method of the invention.

Applicant respectfully requests that the claims of the present application be passed to issue.

The Commissioner is hereby authorized to charge any additional fees which may be required in this application to Deposit Account No. 06-1135.

Respectfully submitted,
Fitch, Even, Tabin & Flannery

James P. Krueger
Registration No. 35,234

Date: MAY 0 3 2005

FITCH, EVEN, TABIN & FLANNERY
120 S. LaSalle St.,Suite 1600
Chicago, Illinois 60603
Telephone (312) 577-7000
Facsimile (312) 577-7007